

WHAT IS CLAIMED IS:

1. A method for identifying inhibitors of neuronal degeneration comprising (a) cotransfecting eukaryotic host cells expressing a presenilin protein (PS), with a polynucleotide encoding a Par-4 polypeptide, and an NF- κ B dependent reporter construct, (b) 5 exposing the cotransfected cells to a candidate molecule, and (c) monitoring the ability of said candidate molecule to induce NF- κ B activation.
2. The method of claim 1 wherein said eukaryotic host cells are mammalian cells endogenously expressing PS.
3. The method of claim 1 wherein said eukaryotic host cells are mammalian 10 cells transfected with nucleic acid encoding PS.
4. The method of claim 3 wherein said PS is PS1.
5. The method of claim 4 wherein said PS1 is human.
6. The method of claim 3 wherein said PS is FAD PS.
7. The method of claim 6 wherein said FAD PS is FAD PS1.
8. The method of claim 7 wherein said FAD PS1 is human. 15
9. The method of claim 1 wherein said eukaryotic host cells are neuronal cells.
10. The method of claim 9 wherein said neuronal cells are cerebellar granule cells.
11. The method of claim 9 wherein said neuronal cells are organotypic brain cells obtained from transgenic mice genetically engineered to express human PS1. 20
12. The method of claim 11 wherein said human PS1 is FAD PS1.
13. The method of claim 1 wherein said NF- κ B dependent reporter construct comprises a luciferase reporter gene.
14. The method of claim 13 wherein said NF- κ B dependent reporter construct comprises NF- κ B-binding consensus sites linked to a luciferase reporter gene.
15. The method of claim 1 wherein the ability of said candidate molecule to 25 induce NF- κ B activation is monitored in comparison with a known inducer of NF- κ B activation.
16. The method of claim 15 wherein said known inducer of NF- κ B activation is TNF- α .

17. The method of claim 1 wherein the cotransfected cells are exposed to a plurality of candidate molecules.

18. The method of claim 1 further comprising the step of administering an identified inhibitor to a patient suffering from or at risk of acquiring a neurodegenerative disease.

19. The method of claim 18 wherein said neurodegenerative disease is Alzheimer's disease.

20. A method for identifying inhibitors of neuronal degeneration, comprising (a) transfecting eukaryotic host cells endogenously expressing Par-4 and a presenilin (PS) protein with nucleic acid encoding an NF- κ B dependent reporter construct, (b) exposing the transfected cells to a candidate molecule, and (c) monitoring the ability of said candidate molecule to induce NF- κ B activation.

21. The method of claim 20 wherein said eukaryotic host cells are HeLa cells.

22. The method of claim 20 wherein the transfected cells are exposed to a plurality of candidate molecules.

23. A method for identifying inhibitors of Par-4 expression or activity comprising (a) transfecting eukaryotic host cells endogenously expressing Par-4 and a presenilin (PS) protein with nucleic acid encoding an NF- κ B dependent reporter construct, (b) exposing the transfected cells to a candidate molecule, and (c) monitoring the ability of said candidate molecule to induce NF- κ B activation.

24. A method for identifying inhibitors of Par-4 expression or activity comprising (a) transfecting mammalian cells with nucleic acid comprising a Par-4 promoter region fused to a reporter gene, (b) exposing said cells to a pro-apoptotic agent followed by exposure to a candidate molecule, and (c) monitoring the ability of said candidate molecule to inhibit the activity of said reporter gene.

25. The method of claim 24 wherein said Par-4 gene is of human origin.

26. The method of claim 24 wherein said reporter gene is a luciferase gene.

27. The method of claim 24 wherein said cell is a cell line endogenously expressing Par-4.

28. The method of claim 27 wherein said cell line is a HeLa cell line.

29. The method of claim 24 wherein said cell is exposed to a plurality of candidate molecules.

30. A method for identifying inhibitors of neuronal degeneration, comprising (a) exposing eukaryotic host cells expressing presenilin (PS) and Par-4 to a candidate molecule, and (b) monitoring the NF- κ B DNA binding activity in the cell extract.

31. The method of claim 30 wherein NF- κ B DNA binding activity is monitored by electrophoretic mobility shift assay.

32. A method for identifying inhibitors of neuronal degeneration, comprising (a) exposing eukaryotic host cells expressing presenilin (PS) and Par-4 to a candidate molecule, and (b) monitoring ξ PKC in the cell extract.

33. The method of claim 32 wherein said ξ PKC is monitored by an enzymatic assay.

34. A method for identifying inhibitors of neuronal degeneration, comprising (a) exposing eukaryotic host cells expressing presenilin (PS) and Par-4 to a candidate molecule, and (b) monitoring the level of I κ B kinase (IKK) phosphorylation.

35. The method of claim 34 wherein the level of I κ B kinase (IKK) phosphorylation is measured by metabolic labeling and immunoprecipitation.

36. The method of claim 35 wherein immunoprecipitation of the cell extract is performed with IKK specific antibodies.

37. A method for identifying inhibitors of neuronal degeneration comprising (a) transfecting a mammalian cell with nucleic acid comprising a Par-4 promoter region fused to a reporter gene, (b) exposing said cell to a pro-apoptotic agent followed by exposure to a candidate molecule, and (c) monitoring the ability of said molecule to inhibit the activity of said reporter gene.

38. An isolated nucleic acid molecule comprising a Par-4 promoter region.

39. The nucleic acid molecule of claim 38 wherein said Par-4 is of human origin.

40. An expression vector comprising a Par-4 promoter region.

41. The expression vector of claim 40 further comprising nucleic acid encoding a heterologous polypeptide under the control of said Par-4 promoter region.

42. The expression vector of claim 41 further comprising nucleic acid encoding a reporter gene under the control of said Par-4 promoter region.

43. The expression vector of claim 42 wherein said reporter gene is a luciferase gene.

5 44. A recombinant host cell transformed with an expression vector comprising nucleic acid encoding a heterologous polypeptide under the control of a Par-4 promoter region.

45. A method for producing a heterologous polypeptide comprising transforming a host cell with nucleic acid comprising the coding sequence of said polypeptide under
10 control of a Par-4 promoter region and culturing the transformed host cell.

46. A method for identifying inhibitors of neuronal degeneration comprising (a) exposing eukaryotic host cells expressing presenilin (PS) and Par-4 to a candidate molecule, (b) exposing said cell to a pro-apoptotic agent, and (c) monitoring ξ PKC in the cell extract.

47. The method of claim 46 wherein said ξ PKC is monitored by an enzymatic
15 assay.

48. A method of inhibiting Par-4 activity in eukaryotic cells comprising introducing into said cells a nucleic acid comprising a Par-4 promoter region.

49. A method of preventing neuronal degeneration in a mammal comprising introducing into said mammal a nucleic acid comprising a Par-4 promoter region.

20 50. A method of preventing neuronal degeneration in a mammal comprising introducing into said mammal an antisense nucleic acid comprising a sequence complementary to a Par-4 promoter region.

51. Inhibitors of neuronal degeneration identified by the method of any of claims 1, 20, 30, 32, 34, 37 and 46.

25 52. A process for obtaining a compound for the treatment of neuronal degeneration in a mammal, said process comprising:

screening a plurality of compounds for their ability to inhibit Par-4 activity; and

preparing a pharmaceutical composition comprising one or more of said compounds identified in the screening and a suitable pharmaceutically acceptable carrier.

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